

# Supporting Information

Cannata *et al.* 10.1073/pnas.0710433105

## SI Text

**In Vitro Cleavage Assay.** We used the pcDNA-T/S plasmid (5900 bp) or a 29-bp-long DNA intramolecular duplex containing the oligopyrimidine–oligopurine target sequence for triplex formation. The DNA substrate was incubated with OP-TFO/LNA and 20  $\mu$ M CuSO<sub>4</sub> in 50 mM Hepes (pH 7.5), 10 mM MgCl<sub>2</sub>, 50 mM NaCl; the cleavage reaction was started by addition of 3 mM MPA (3-mercaptopropionic acid) and performed at 37°C. The reaction was stopped by adding 250  $\mu$ M 2,9-dimethyl-1,10-phenanthroline (neocuproine).

The supercoiled pcDNA-T/S plasmid was linearized with the restriction enzyme SacII after the cleavage reaction. The cleavage products were analyzed on 0.8% agarose gels (TAEx1) stained with ethidium bromide. The 29-bp duplex was end-labeled by using T<sub>4</sub> polynucleotide kinase and  $\gamma$ -[<sup>32</sup>P]ATP. Cleavage products were analyzed on 15% denaturing polyacrylamide gel (TBEx1).

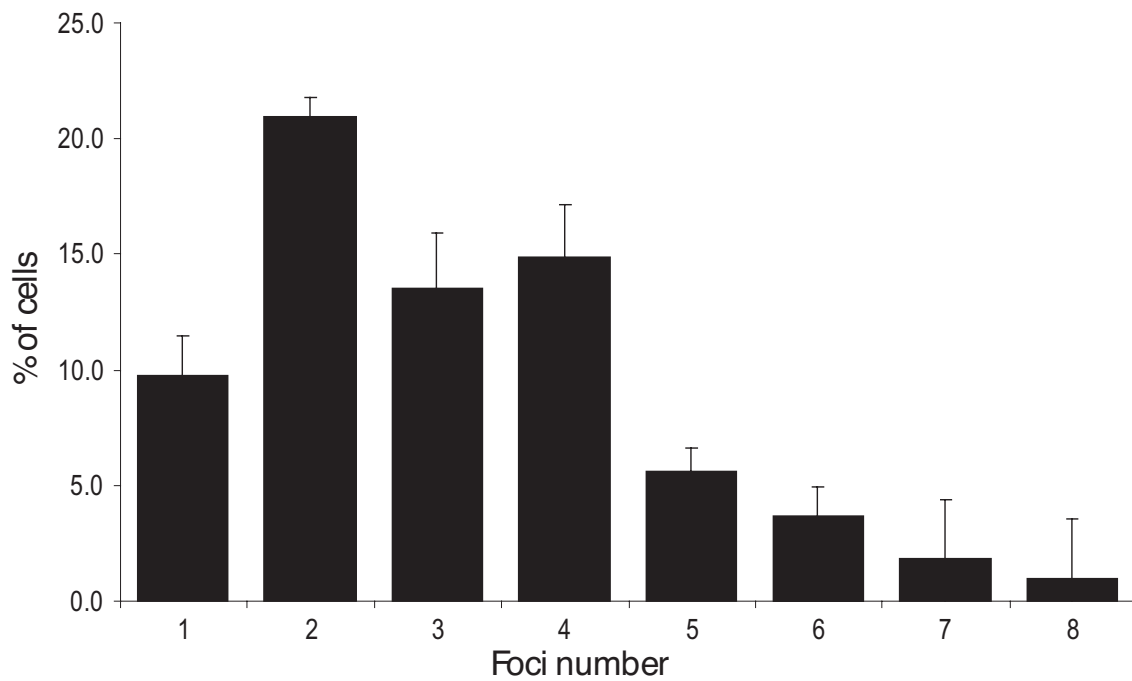
OP-TFO/LNA transfection into HeLa/luc cells and immortalized fibroblasts was performed by streptolysin O-mediated permeabilization (Fig. S2). These two cell lines were described in refs. 22 and 28, respectively. Streptolysin-O (SLO) (a gift from S. Bhakdi, Johannes Gutenberg-Universität, Mainz, Germany) was used to reversibly permeabilize these two cell lines. The protocol used is a modification of a previously described SLO-based method (35). Briefly, cells were washed twice in HBSS without Ca<sup>2+</sup> (GIBCO), and 2  $\times$  10<sup>6</sup> cells were resuspended in 100  $\mu$ l of HBSS. An optimized amount of SLO and 10  $\mu$ l of OP-TFO/LNA (100 $\times$ ) were added for a 15-min incubation at 37°C to allow SLO-induced permeabilization. DMEM (0.9 ml) containing 1.8 mM Ca<sup>2+</sup> were then added to reseal cells.

**Chromatin Immunoprecipitation.** Confluent monolayers of HeLa/luc cells were fixed with formaldehyde (1% vol/vol) for 10 min at 37°C. Cross-linking was stopped by addition of glycine to a final concentration of 0.125 M. Cross-linked cells were harvested and washed in PBS. Subsequent procedures were performed on ice, with buffers supplemented with protease inhibitor mix (Roche Diagnostics). Cells were lysed in lysis buffer (5 mM Pipes (pH 8.0), 85 mM KCl, 0.5% Nonidet P-40) and incubated on ice for 10 min (with magnetic stirring). Nuclei were pelleted and lysed by incubation in nuclear lysis buffer [50 mM Tris-HCl (pH 8.1), 10 mM EDTA, 1% SDS]. Sonication was performed with a Bioruptor type sonicator (Diagenode) in closed 1.5-ml tubes. Settings of the sonicator were: first cycle (15 min) at medium intensity: 20 sec ON/ 1 min OFF; second cycle (15 min) at high intensity: 10 sec ON/ 1 min OFF; third cycle (6 min) at high intensity: 20 sec ON/1 min OFF.

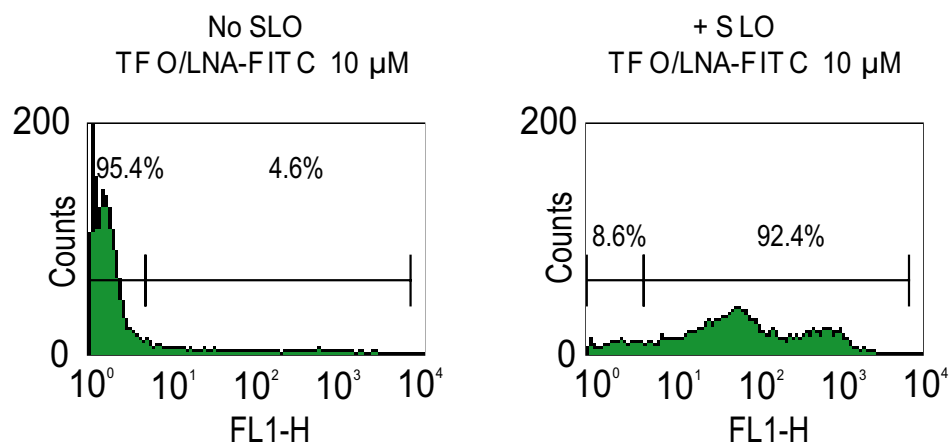
DNA contents in nuclear extracts were standardized after quantification by using a NanoDrop UV-Vis. spectrophotometer. After centrifugation, the supernatant was diluted 10-fold with dilution buffer [0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM TrisHCl (pH 8.1), 167 mM NaCl]. Diluted extracts were precleared with protein A/protein G agarose beads (Sigma) and incubated with anti-phosphoH2AX antibodies (Upstate), and immunoprecipitated with protein A/protein G agarose beads (Sigma). After extensive washing, bound DNA fragments were eluted by overnight incubation at 65°C and purified by treatment with proteinase K, phenolchloroform extraction, and ethanol precipitation. Samples were analyzed by quantitative PCR (Mx3005P Real-Time PCR System Stratagene) using specific primers (see sequences in Table S1).

1. Porteus MH, Carroll D (2005) *Nat Biotechnol* 23:967–73.
2. Alwin S, *et al.* (2005) *Mol Ther* 12:610–7.

3. Bibikova M, Golik M, Golik KG, Carroll D (2002) *Genetics* 161:1169–75.



**Fig. S1.** Detailed quantification of phosphoH2AX foci number 24 h after OP-19-mer TFO/LNA treatment (corresponding to data from Fig. 2A *Right*).



**Fig. S2.** Evaluation of transfection efficiency of OP-TFO/LNA conjugates. FACS analysis of cells was performed 1 h after SLO permeabilization of HeLa/luc cells in presence of 10  $\mu$ M TFO/LNA-FITC. Dual-parameter flow cytometry using propidium iodide (added just before FACS analysis), for dead-cell staining, and the fluorescein-labeled oligonucleotide, TFO/LNA-FITC, for monitoring transfected cells, was performed. Transfection efficiency of fluorescein-labeled oligonucleotide was  $\approx 90\%$ . Fluorescence microscopy allowed the observation of uniform distribution of TFO/LNA-FITC within cells (data not shown).

A

### HeLa Cells

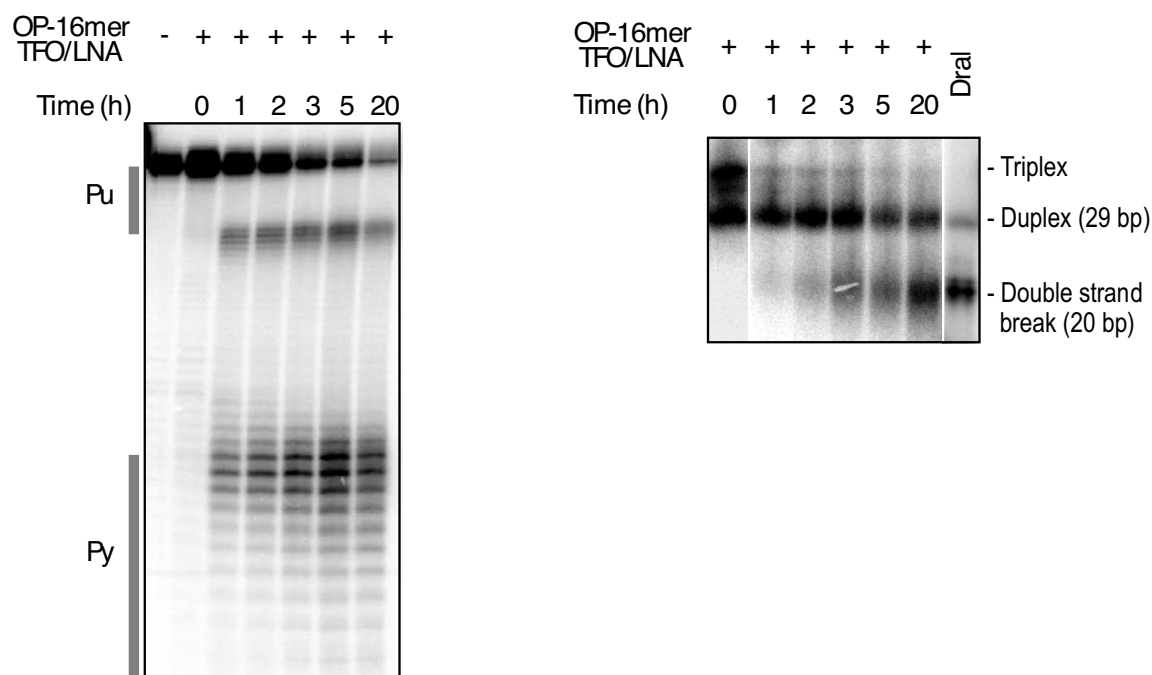
		-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
Untreated		T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	86	100.0%					
Experiment A	Experiment A	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	78	85.7%					
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
Experiment B	Experiment B	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	75	86.2%					
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	2	2.3%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.1%						
Experiment C	Experiment C	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	62	89.9%					
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						
	T	A	T	G	A	G	A	G	A	C	A	A	G	T	C	T	T	T	A	T	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	A	A	T	G	A	A	T	1	1.4%						

B

### Immortalized Fibroblast

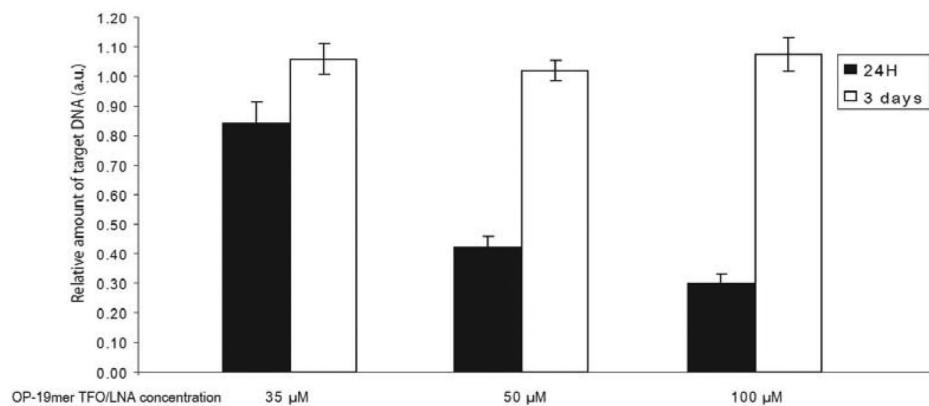
[illegible]

**Fig. S3.** Mutations patterns 72 h after treatment with OP-19-mer TFO/LNA (50  $\mu$ M during the SLO-permeabilization procedure). Three independent experiments were performed in HeLa cells (A–C) and in human immortalized fibroblasts (D to F), respectively. These data correspond to the histogram presented in Fig. 3C. The OP-19-mer TFO/LNA target sequence is indicated in bold.

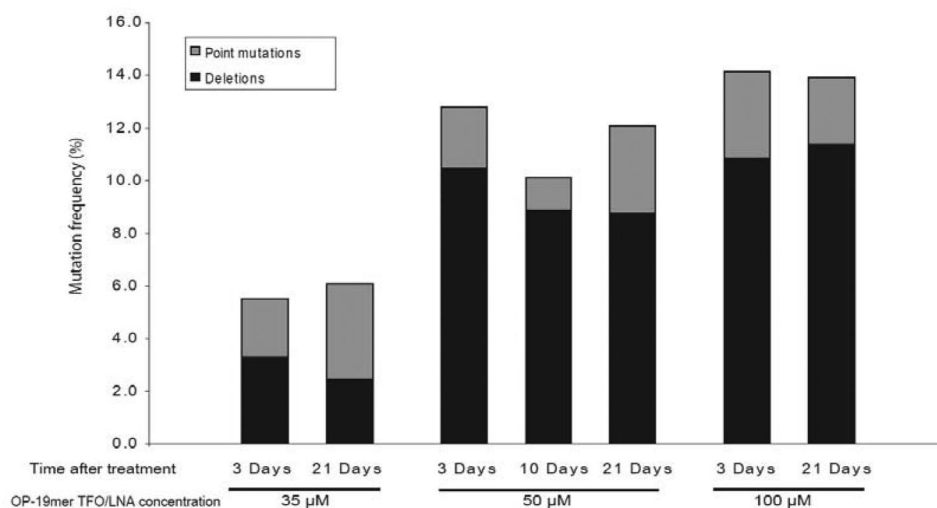


**Fig. S4.** Cleavage activity of OP-16-mer TFO/LNA conjugate on a synthetic DNA target. An intramolecular duplex (29 bp) was used as a target after 5' radiolabeling. Cleavage products were analyzed by denaturing (*Left*) or nondenaturing (*Right*) PAGE. A schematic representation of the corresponding cleavage pattern is depicted in Fig. 1 in the main text.

**A**

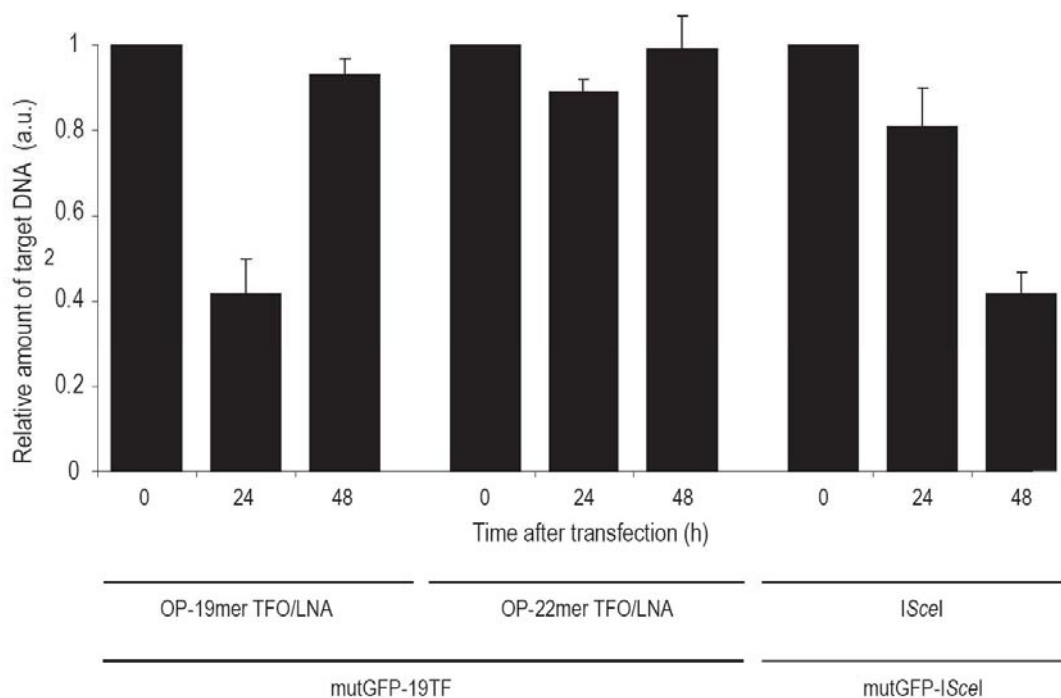


**B**



**Fig. S5.** Dose-dependent activity of OP-19-mer TFO/LNA. (A) Dose-dependent induction of DSBs at the target site. PCR quantification of the target DNA was performed by using primers flanking the OP-19-mer TFO/LNA-binding site, at the indicated times after treatment of HeLa/mutGFP-19TF cells with the indicated concentration of OP-19-mer TFO/LNA conjugate. The values were normalized by quantification of a control genomic sequence where no triplex site is available. (B) Dose-dependent induction and long-term stability of mutations at the target site. The target region was amplified at 3, 10, and 21 days after treatment of HeLa/mutGFP-19TF cells with the indicated concentration of OP-19-mer TFO/LNA conjugate and individual PCR products sequenced after cloning. (C) Sequencing results. The indicated concentrations correspond to the ones used during the SLO permeabilization procedure (see *Materials and Methods*).

		-10	-9	-8	-7	-6	-5	-4	-3	-2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
OP-19mer TFO/LNA	3 Days	Untreated	T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	65	100.0%	
		T	C	C	A	A	G	C	T	T	T	.	.	A	A	A	G	A	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	86	94.5%
		T	C	C	A	A	G	C	T	T	T	.	.	A	A	A	G	A	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.1%
		T	C	C	A	A	G	C	T	T	.	.	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.1%
		T	C	C	A	A	G	C	T	T	.	.	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.1%
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.1%
	21 Days	T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
OP-19mer TFO/LNA 50 μM	3 Days	T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	75	87.2%		
		T	C	C	A	A	G	C	T	T	T	A	A	A	A	G	A	A	A	A	A	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%		
		T	C	C	A	A	G	C	T	T	T	.	.	A	A	A	G	A	A	A	A	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%		
		T	C	C	A	A	G	C	T	T	T	.	.	A	A	A	G	A	A	A	A	A	G	G	G	G	G	A	G	A	G	C	A	A	A	T	T	T	G	T	A	1	1.2%	
		T	C	C	A	A	G	C	T	T	T	.	.	A	A	A	G	A	A	A	A	A	G	G	G	G	G	A	G	A	G	C	A	A	A	T								





**Table S1. Primer sequences**

Primer sequence (5'–3')	Amplified region	Amplicon size, bp
CTGCCTGCAGGAGAAAAGAA	Chr 7 around the OP-19-mer TFO/LNA target sequence (for qPCR)	191
CCCTCCCATTTCAAAGTCAG		
TGATAATAGAGCCAATCCCTCA	Chr 7 around the OP-19-mer TFO/LNA target sequence (for junction analysis)	405
TTTGTCCCATTTCTGTTTTATTCA		
TCCCACCTTCTGAAAAGTCTGTACT	Chr 7, 3 kbp from the OP-19-mer TFO/LNA target sequence (for ChIP)	172
AAAGAGAAACTGAGAGGAAAAATCA		
CCACTCCTGATTTCTGGAAAAG	GAPDH (for ChIP)	171
GAAATTAAGTGGACAGGGCAAG		
ATGGTGAGCAAGGGCGAG	Integrated mut eGFP (for sequence analysis)	513
GACGTTGTGGCTGTTGTAGTTG		
CCTCGTGACCACCTGAC	Integrated mut eGFP (for qPCR)	107
GGTAGCGGCTGAAGCACT		
ACATGAAGCAGCAGCACT	Reference region (for qPCR)	93
TCTTGTAGTTGCCGTCGT		